

INTERACTIVE EFFECTS OF NUTRIENTS AND  
TEMPERATURE ON PLANT-HERBIVORE  
INTERACTIONS

By

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#### Abstract

Global environmental changes such as increased temperatures and deposition of nutrients are greatly affecting many organisms, including a number of Lepidoptera species. Numerous studies have been conducted to determine the impacts of temperature and nutrients on herbivores, although many have tested each variable separately and could consequently be missing possible interactive effects. In this experiment, we tested the interactive effects of nutrients and temperature on interactions between *Pieris rapae* and its host plant, *Brassica oleracea*. Using a full-factorial design, we grew Brussels sprouts at one of three temperatures ( $16\pm 2^{\circ}\text{C}$ ,  $23\pm 2^{\circ}\text{C}$ , or  $30\pm 2^{\circ}\text{C}$ ), on one of three nutrient treatments that varied in the ratio of nitrogen to phosphorus (4:1, 16:1, or 64:1). One first instar caterpillar was assigned to each plant at the various treatments and was fed experimental leaves until pupation. We measured macronutrient and elemental content of leaves for each plant, as well as various life history traits for larvae, including time to pupation, amount of leaves eaten, adult mass, and adult lipid content. Plant macronutrient content was not affected by temperature treatment, but was significantly affected by nutrient treatment. We found an interactive effect for only one variable - time to pupation - with the majority of other significant variables being influenced by temperature, such as time to pupation, survival, and adult size. We also found adult lipid content to be highly affected by nutrient treatment, with butterflies from the warmest treatment having a higher lipid content. Our results suggest that temperature may be the most influential variable for herbivore growth and fitness.

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## CHAPTER I

### INTERACTIVE EFFECTS OF NUTRIENTS AND TEMPERATURE ON PLANT- HERBIVORE INTERACTIONS

#### Introduction

Human-induced environmental changes, including global climate change and increased nutrient inputs, are having a large impact on the distributions and abundances of many species; some species are negatively impacted from these changes, while others, such as pest species, are greatly benefiting (Sih et al. 2001). Warming temperatures have affected the phenology of many bird, butterfly, and plant species, and in general spring activities including breeding and first appearance have been occurring increasingly earlier since the 1960s (Walther et al. 2002). There has also been a shift in species ranges, with a poleward and upward range shift occurring across a wide range of taxonomic groups (Hughes 2000; McCarty 2001). Climate change has increased voltinism, the number of reproductive generations in a year, in many herbivorous insects, particularly lepidopterans (Altermatt 2010). An increase in voltinism can cause outbreaks of herbivorous pests of the agriculture and forestry industries (Porter et al. 1991; Steinbauer et al. 2004). While it is known that anthropogenic environmental change is affecting species in many ways, less is known about the mechanisms through which human actions impact trophic interactions.

Global climate change is expected to affect plant-herbivore interactions through both direct and indirect means (DeLucia et al. 2012). Temperature is one of the most influential

environmental factors that affects growth and biological processes in organisms (Lee and Roh 2010). Studies of herbivores have shown that increased temperature directly results in shorter development time, and decreased body mass (Lee and Roh 2010; Bauerfeind and Fischer 2013). Temperature also indirectly affects herbivores by decreasing the quality of host plants on which they feed by altering the amount or ratio of nutrients in plant tissues (DeLucia et al. 2012). For example, a review by Zvereva and Kozlov (2006) found that when combined with increased carbon dioxide – another expected result of climate change – increased temperature decreased the nitrogen content and C:N ratio in plants, and increased leaf toughness. As a result of altered plant quality, herbivores feeding on plants grown at high temperatures have longer pupal development time, lower adult mass, reduced digestive efficiency, and increased food consumption (Bauerfeind and Fischer 2013). Reduced digestive efficiency and increased food consumption suggest that herbivores are exhibiting compensatory feeding as a mechanism to ameliorate poor plant quality.

Host plant quality can also be influenced by increased nutrient deposition. There has been a significant increase in nitrogen deposition to ecosystems due to agricultural runoff and fossil fuel combustion (Vitousek et al. 1997). The environmental consequences of increased nutrient inputs have been most well studied in aquatic systems where it results in eutrophication. However, atmospheric deposition and runoff of nutrients can also have important impacts on terrestrial systems by affecting the concentrations of nutrients in plants (Smith et al. 1999). As with temperature, increased nutrients can affect plant quality, and thus the herbivores that feed on them. N has an essential role in building tissues and is thought to be a limiting nutrient for herbivores (Mattson 1980). In addition to nitrogen, phosphorus deposition is also occurring, primarily through agricultural runoff. Phosphorus is an important element for herbivores, and its limitation affects many of the same life history traits that are influenced by temperature (Tao and

Hunter 2012). The ratio of N:P can have important consequences for biochemical processes in plants and animals depending on the rate at which these nutrients are stored versus allocated to biological molecules. Yet the effects of elemental supply on macronutrients, the nutrients that herbivores regulate in their diet, remains poorly understood (Perkins et al. 2004).

Many studies have investigated the separate impacts of temperature and nutrients on plant-herbivore interactions. For example, previous studies have grown plants and caterpillars at a range of temperatures in separate environments, then fed caterpillars the nutritionally manipulated leaves and studied the effects on the caterpillars' life history (Bauerfeind and Fischer 2013), or reared caterpillars at increased temperatures while feeding them nutritionally manipulated artificial diets (Lee and Roh 2010). Other studies have demonstrated the importance of larval nutrition on adult fitness without examining how these relationships are affected by temperature (Boggs 2009; Tigreros 2013). A recent review suggested that there may be strong interactive effects of temperature and nutrients on life history traits and trophic interactions (Cross et al. 2015). However, to date there are very few studies that have investigated these interactions together in one system. This presents a significant knowledge gap, as these factors will realistically be occurring at the same time and are likely to have interactive effects.

In this study, we tested the interactive effects of nutrients and temperature on plant-herbivore interactions between Brussels sprouts, *Brassica oleracea*, and cabbage white butterflies, *Pieris rapae*. Our objective was to provide a comprehensive test of how nutrient-temperature interactions affect plant-herbivore interactions, and the mechanisms responsible for these effects in a single study system, starting with nutrients in the soil for plants and ending with adult life stage of herbivores. Climate change is predicted to have negative consequences for plant-herbivore interactions in the form of higher levels of herbivory and lower herbivore growth, but some of these effects could be altered by nutrient additions to soil. Climate change will directly affect plants through increased temperatures and carbon dioxide, but can also have

indirect impacts through population dynamics of pest herbivores (Gilman et al. 2010). The results of this study have important implications for management of both endangered and pest Lepidoptera and other insect herbivores in response to climate change.

We addressed two main questions: 1) How do soil nutrients and temperature interact to affect plant growth and composition in Brussels sprouts? 2) How do nutrient and temperature treatments and their effects on plant quality affect *P. rapae* herbivory rates, caterpillar growth, and adult body size? We hypothesized that caterpillars reared on the treatment at the higher temperature (30°C) would show significantly higher growth on low levels of N:P (4:1) than the other nutrient treatments, as high temperatures allow rapid growth and protein availability may be a major factor limiting growth. High temperatures often result in smaller body size in many herbivores potentially because protein is not available at high enough rates to sustain large body size. Preliminary work also suggests that protein availability may be highest in plants fertilized with low N:P (Wilder and Jeyasingh unpublished data). We also hypothesized that caterpillar growth would be similar among the nutrient treatments at the lower temperature because the overall slower growth rate on these treatments will mean that nutrient supply rate is relatively less important than the rate at which nutrients can be used for growth. Lastly, we hypothesized that, in general, plant quality would decrease with increasing temperature as has previously been demonstrated, but that some of these effects could be ameliorated by the nutrient treatment on which the plant is grown.

## Methods

### Study System:

*Brassica oleracea*, whose naturally occurring form is known as wild cabbage, is a plant species that includes many cultivars that are economically important to agriculture, including cabbage, kale, broccoli, and Brussels sprouts. We used Nautic F1 organic certified Brussels sprout seeds from West Coast Seeds (Delta, BC, Canada) in this experiment. Plants were grown in a controlled soil mixture with a 2:2:1:1 ratio of peat moss: vermiculite: sand: soil. This mixture has very low nutrient content, which allowed us to experimentally control nutrient availability for plants.

Almost all plants in the Brassicaceae family contain glucosinolates in their tissues, which is a class of secondary defense compounds. The glucosinolate-myrosinase complex is a two-component system and the negative effects of these secondary compounds are activated when plant tissue is damaged and glucosinolates come into contact with plant myrosinase, which is stored separately in the plant (Matile 1980; Bones and Rossiter 1996). When these two compounds are combined, they form isothiocyanates through a hydrolysis reaction, which are toxic to many herbivores (Wittstock et al. 2003; Agrawal and Kusrashige 2003). However, *P. rapae* is a specialist on brassica plants and biochemically counters this defense system by redirecting the hydrolysis towards the formation of less toxic nitriles which are excreted in the frass, therefore circumventing the formation of toxic isothiocyanates (Wittstock et al. 2004). Because of this counteradaptation that allows *P. rapae* to be mostly unaffected by the secondary defenses in Brussels sprouts, we did not measure glucosinolate content in leaves.

*Pieris rapae*, commonly known as the cabbage white butterfly, is native to Europe, Asia, and North Africa. It has been introduced throughout North America, Australia, and New Zealand, where it is an invasive species. Throughout its range, it is an agricultural pest of wild and

cultivated plants in the Brassicaceae family. Caterpillars used in this experiment came from an established laboratory colony of cabbage white butterflies that were purchased from Carolina Biological Supply, which had been maintained in the laboratory for over three years. The colony was maintained at  $\sim 25^{\circ}\text{C}$  and eggs were laid on Brussels sprout plants grown using the controlled soil mixture with the 16:1 N:P and added potash and azomite. After hatching, caterpillars were reared on a stonefly heliothis artificial diet (Ward's Science).

To determine the effects of soil nutrients and temperature on plants, we used a full factorial design to grow Brussels sprouts at one of three temperatures ( $16 \pm 2^{\circ}\text{C}$ ,  $23 \pm 2^{\circ}\text{C}$ , or  $30 \pm 2^{\circ}\text{C}$ ) combined with one of three soil nutrient treatments that varied in the ratio of nitrogen to phosphorus (4:1, 16:1, or 64:1), for a total of 9 treatments. The experimental temperatures and nutrient treatments were chosen based on previous studies of *P. rapae* and preliminary data (Kingsolver 2000; Kingsolver et al. 2006; Wilder unpublished data). Brussels sprouts seeds were first sprouted on moist paper towels, then transferred to a 470ml plastic cup that contained 120 mL of the controlled soil mixture, combined with one of the nutrient treatments ( $n=18/\text{trt}$ ). To water the plants, the plastic cups were placed on top of a larger plastic cup that contained water, and a cotton wick was used. Seedlings were then placed in the assigned temperature treatment.

#### Temperature Manipulations:

We conducted all temperature manipulations in the same controlled environmental chamber. We constructed three 63.5 x 73 x 42cm boxes using thermal insulating material, with a door on the front to allow opening and closing of the box, and each box was placed on a separate shelf in the environmental chamber. To achieve the desired temperature for the treatment, we used heating cords on timers placed on the bottom of each box. Within each box, temperatures fluctuated on a daily cycle. Temperatures were at their highest value ( $18^{\circ}\text{C}$ ,  $25^{\circ}\text{C}$ , and  $32^{\circ}\text{C}$ )

during the day and their lowest value (14°C, 21°C, and 28°C) during the night to simulate natural temperature fluctuations. Treatments are identified by their daily mean temperature (i.e., 16, 23 or 30°C). A fluorescent light (Pioneer Jr. high output T5 fixture) was placed over a rectangular opening cut on the top of each box to provide light for the plants. Lights were on a 14:10 hour light dark cycle.

#### Soil Nutrient Manipulations:

Nutrient ratios were calculated by keeping the total mass of N and P atoms constant, and altering the amounts of each nutrient to achieve the differing ratios of N:P. We used powdered ammonium nitrate as a nitrogen source and powdered triple super phosphate as a phosphorus source. The amounts for each nutrient ratio are as follows: 4:1 - 0.706g NH<sub>4</sub>NO<sub>3</sub>: 0.133g phosphate; 16:1 - 0.830g NH<sub>4</sub>NO<sub>3</sub>: 0.039g phosphate; 64:1 – 0.869g NH<sub>4</sub>NO<sub>3</sub>: 0.010g phosphate. The intermediate 16:1 is the classic optimal balance of N:P for aquatic systems and is close to the ratio of about 13:1 that has been reported in terrestrial systems (Cleveland and Liptzin 2007).

Each nutrient treatment was supplemented with powdered potash (K<sub>2</sub>O) and 0.15g of azomite 0-0-0.2 (0.2% soluble K<sub>2</sub>O, 1.8% Ca, 0.5% Mg, 0.1% Cl, 0.1% Na) to prevent possible deficiencies of other trace elements. To maintain similar pH levels for each treatment (~7), we added 0.055g of potash to the 16:1 and 64:1 treatments, and 0.077g to the 4:1 treatment. Nutrients were mixed with 120mL of controlled soil mixture prior to planting sprouts.

### Feeding Setup:

When each plant had four fully expanded full leaves, we harvested the third leaf – starting from the bottom of the stem – from each plant to measure the macronutrient and elemental content, and the remaining leaves were used for caterpillar experiments. For sample leaves, we recorded wet mass, length and width measurements, and dry mass after drying in an oven at 60°C for 24 hours. Newly hatched (3-5 days post hatching) first instar *P. rapae* caterpillars were assigned to each treatment, with one caterpillar assigned per plant (n=18/trt). All applicable institutional and/or national guidelines for the care and use of animals were followed. A leaf was cut from each plant and an initial wet mass and length and width measurements were taken. Because leaves needed to be completely flattened in order to be measured using Image J software, we took initial leaf measurements using a paper ruler so as not to damage leaves prior to feeding to caterpillars. We then inserted the stem of the leaf into a hole drilled into the side of an inverted tube filled with water to prevent wilting of the leaf during feeding to caterpillars. We placed the leaf and water tube in a 15 x 12 cm clear plastic container and one first instar caterpillar was placed on the leaf. The container with the leaf and caterpillar was placed in the assigned temperature treatment in the environmental chamber. We removed and replaced the leaf when it was almost entirely eaten or after five days. Caterpillars were checked daily. After removal of the eaten leaf, we took a digital image of each one to analyze the leaf area consumed using Image J software. We used the following equation to convert the initial leaf measurements taken using the ruler to the final measurements taken using Image J software:

$$y = 1.0301x - 2.7535$$

Leaves were provided to caterpillars until pupation.

For each caterpillar, we measured the time to pupation in days, pupal duration in days, and total leaf area eaten in cm<sup>2</sup>. We also collected frass during the third and fourth instars and



measured its mass and elemental content. Within a day of emergence and after their wings were completely dry, adult butterflies were placed in a freezer to be euthanized. We then recorded the wet mass and sex of each adult butterfly, measured total wing area using Image J software, and measured lipid content of the bodies.

To estimate the dry mass of leaves eaten by caterpillars, we created a regression using the relationship between leaf area of the sampled third leaf, which was taken using the paper ruler, and sample leaf dry mass, then input the leaf area eaten by caterpillars to determine the mass of leaves eaten. We estimated conversion efficiency for caterpillars by dividing adult dry mass by the estimated dry mass of leaves eaten.

#### Nutrient Assays:

For assays, the third leaf from each plant and the frass collected during the third and fourth instars were dried in an oven at 60°C for 24 hours. We then finely ground and homogenized each leaf and frass sample, and used a new subsample for subsequent analyses.

***Elemental Content*** – We measured the C and N content of plant tissue and caterpillar frass by subsampling 2-5 mg of dry leaf tissue and 2-5 mg of dry frass, packaging it in a tin capsule, and analyzing it in a CN analyzer (varioMicro Cube, Elementar Americas, Inc.).

***Macronutrients*** – We measured leaf carbohydrate content using a sulfuric acid – UV spectrophotometry assay (Albalasmeh et al. 2013). We subsampled 3-5 mg of dry leaf tissue and extracted carbohydrates by adding 0.2N sulfuric acid to each sample, boiling for one hour,

centrifuging, then removing the supernatant. We set up a standard curve using stock glucose solution, water, and concentrated sulfuric acid. For the assay, we added water and concentrated sulfuric acid to 30 $\mu$ L of each sample, then read each in a BioMate 3S UV-Visible spectrophotometer and recorded the absorbance. We then calculated carbohydrate concentration and percentage.

Leaf protein content was measured using the Bradford Assay. We subsampled 3-5 mg of dry leaf tissue and solubilized the proteins with 0.1M sodium hydroxide, then heated, sonicated, and centrifuged the samples, and removed the supernatant. We purified the samples to remove any compounds that would interfere with the assay using HCl and TCA. For the assay, 10 $\mu$ L of each purified sample was added to a well plate with water and dye reagent, and read on a Multiskan FC microplate reader. We took the average fitted concentration of three replicates for each sample to account for possible errors in pipetting and calculated percent protein.

We measured lipid concentration of the adult butterfly bodies using chloroform extraction (Wilder et al. 2013). We recorded an initial dry mass for each butterfly body, then placed each in a glass vial and filled it halfway with chloroform. The vials were sealed and after 24 hours the chloroform was removed and the process was repeated once more. We dried the samples at 60°C for 12 hours and recorded a final dry mass, then used the change in mass to determine the percent lipid of each sample.

#### Analysis:

Data were analyzed as two factor analysis of variance (ANOVA) using JMP statistical analysis software.

## Results

### Plants:

There was no effect of nutrient or temperature treatment on the macronutrient composition of Brussels sprout leaves (Table 1). Leaf protein (Figure 1) and carbohydrate (Figure 2) content did not significantly differ between treatments. There was a significant effect of nutrient treatment on the percentage of nitrogen (Table 1). Plants in the 4:1 treatment had a significantly lower percentage of nitrogen in their leaves compared to those in the 16:1 and 64:1 treatments (Figure 3). There was no effect of nutrient or temperature treatment on leaf carbon (Figure 4), or any interactive effects.

### Caterpillars:

Over the course of the experiment, 102 *P. rapae* were raised from first instar to adults. There was a significant effect of temperature on survival ( $\chi^2 = 40.3$ ,  $p < 0.001$ ). Overall, caterpillars in the 16°C temperature treatment had a higher rate of survival to emergence as butterflies, with 50 of 51 surviving among the three nutrient treatments (Figure 17). In the 23°C treatment, 38 of 41 survived to emergence. The 30°C had a very low survival rate, as well as a low plant growth rate, with only 10 of 23 caterpillars surviving to emergence as butterflies. Of the caterpillars from this temperature treatment that pupated successfully, six did not emerge from the chrysalis. There did not appear to be an effect of diet on survival.

There was no significant effect of nutrient or temperature treatment on the area (Figure 6) or dry mass (Figure 7) of leaves eaten by caterpillars. There was a trend for a nutrient effect, with caterpillars tending to eat more on the 4:1 treatment and less on the 64:1 treatment, but this effect was not strong (Table 2).

However, there were significant effects of temperature on pupation (Table 2). Time to pupation was significantly affected by temperature, with caterpillars at lower temperatures taking more days to pupate than those at higher temperatures (Figure 9). There was also a significant interaction between nutrients and temperature. At lower temperatures, nutrient treatment had a significant effect on time to pupation; caterpillars at 16°C on the 64:1 nutrient treatment took longer to pupate than those on 4:1 and 16:1 at the same temperature (Figure 9). But at higher temperatures, there was no effect of diet on time to pupate. Pupal duration was also significantly affected by temperature (Figure 10, Table 2), with caterpillars at lower temperatures taking longer to emerge. There was no effect of nutrient treatment or interactive effects on pupal duration.

There was a highly significant effect of temperature on conversion efficiency (Figure 8, Table 2), with caterpillars in the 16°C treatment having a higher efficiency than those in the warmer treatments. There was no effect of nutrient treatment or any interactive effects.

There was a significant effect of both nutrient and temperature treatment on the percentage of nitrogen in caterpillar frass (Table 2). Frass from caterpillars in the 16°C treatment contained more nitrogen than frass from caterpillars in the 30°C treatment (Figure 11). While nitrogen content was similar among nutrient treatments at 23°C, at low and high temperatures, frass from caterpillars on the 64:1 nutrient treatment contained more nitrogen than frass from those on the 4:1 treatment. There was no effect of treatment on the percentage of carbon in frass (Figure 12).

Adult dry mass was significantly affected by temperature (Table 2). Butterflies were heavier at lower temperatures than at higher temperatures (Figure 14). Butterflies at higher temperatures also had a larger wing area (Figure 15). There was no effect of nutrient treatment or interaction between nutrients and temperature.

There was a highly significant effect of nutrient treatment on adult body lipid composition (Table 2). Lipid composition was significantly higher in butterflies on the 64:1 treatment (Figure 16). There was almost a significant effect of temperature, with lipid composition tending to be higher at higher temperatures. There was no interactive effect.

## Discussion

The results of this experiment did not support the hypothesis that there would be strong interactive effects of temperature and nutrients. Overall, we found only one temperature x nutrient interactive effect, time to pupation. Temperature was the most influential variable, with the majority of the significant results being affected by temperature. Pupation was highly influenced by temperature; both time to pupation and pupal duration occurred significantly shorter as temperature increased. Butterfly size was also significantly influenced by temperature, with adults being heavier and having greater wing area at lower temperatures. We found a significant nutrient effect for time to pupation, percentage of nitrogen in caterpillar frass and Brussels sprouts leaves, and a highly significant effect for percent lipid composition of adults. It is possible the results did not support our hypothesis because of the potential adaptive capabilities of *P. rapae*. As an invasive pest species, it is possible that this species is better able to compensate for nutrient changes by changing their physiology, while, being an ectotherm, they are unable to change their response to temperature.

The time it took caterpillars to pupate was significantly affected by temperature, nutrient treatment, and an interaction between the two variables. Caterpillars at 16°C took significantly less days to pupate than those at 23°C or 30°C: on average 17-20 days at 16°C, compared to about 10 days and 6 days, respectively, for the warmer treatments. At 16°C there was also an interactive effect, with caterpillars fed leaves fertilized with a 4:1 N:P taking less time to pupate compared to those fed leaves with a 64:1 N:P. However, this interaction did not hold at the warmer temperature treatments. A possible explanation for this trend is conversion efficiency, and the physiological costs for ectotherms associated with both fast growth and fueling metabolic rate in higher temperatures. Studies of vertebrate nutrition have shown that as the quantity of metabolizable – or assimilated – energy ingested by an organism increases, metabolic heat production increases exponentially; therefore, increased energy intake results in a reduced

efficiency in retaining energy (Blaxter and Boyne 1978). Our results support other studies of ectotherms which have found that digestion is less efficient at warmer temperatures compared to colder temperatures. For example, in a study using locusts, individuals exposed to temperatures of 38°C were less efficient at digesting macronutrients than those at 32°C (Miller et al. 2009). It is possible that because of the faster growth rate and increased metabolism, caterpillars at the higher temperatures were unable to digest N and P as efficiently compared to those at the colder temperature, which exhibited slower growth, and therefore a nutrient effect was unnoticeable at 23°C and 30°C. The estimated conversion efficiency supports this; caterpillars at colder temperatures had a higher estimated efficiency than those at warmer temperatures.

We found that temperature treatment had the greatest influence on the results of this experiment, influencing a large proportion of the variables that were significant. In addition to time to pupation, pupal duration was highly significantly affected by temperature, along with adult mass and adult wing area. Caterpillars reared at lower temperatures were heavier as adults and had a larger wing area than those reared at higher temperatures. These findings are consistent with those of other studies of Lepidoptera (Diamond and Kingsolver 2009; Bauerfeind and Fischer 2013), and follow the temperature-size rule (TSR), where lower development temperatures result in larger body size in ectotherms. However, unlike the findings of Diamond and Kingsolver (2009), we did not find support that this relationship was influenced by diet, nor an interaction between diet and temperature. Additionally, we did not find a significant effect of temperature on consumption rate, with caterpillars eating similar amounts at the same nutrient treatments for each temperature treatment. However, a nutrient effect was nearly significant ( $p = 0.07$  for leaf area eaten,  $p = 0.06$  for dry mass eaten). These results are contrary to other studies, which have shown higher consumption rates as metabolism increases with increasing temperature.

Caterpillars at the higher temperature treatments had faster growth, but also had a higher rate of pupation failure, as has been found in studies of other caterpillar species (Kingsolver et al. 2006; Lee and Roh 2010; Lee et al. 2015). Nearly all caterpillars in the 16°C treatment pupated and emerged successfully, while many reared at 30°C either did not pupate properly or failed to emerge after pupation. This trend was more common for larvae in the 4:1 and 64:1 nutrient treatments. Given the large effect of temperature on survival, our results suggest that lower temperatures might result in higher fitness for *P. rapae*.

Lipid content of adults was significantly affected by nutrient treatment. Caterpillars reared on the 64:1 treatment had a higher lipid composition as adults, while those on the 4:1 treatment had a lower composition. There was also a trend for higher lipid composition at higher temperatures. A study with *S. exigua* caterpillars found similar results for temperature treatment, showing that lipid storage efficiency was lower in caterpillars at 18°C than at 26°C, which the authors suggest could be due to an increase in total energy expense associated with longer pupal duration (Lee and Roh 2010). Lee et al. (2015) found that macronutrient balance was a key factor influencing the relationship between temperature and body size in ectotherms, with the nutrient-dependent effects of temperature on body size resulting from corresponding changes in body lipid storage. In their study, the authors found that lipids were stored less efficiently at higher temperatures, but this effect could be buffered by diet, with caterpillars choosing to eat more carbohydrates when reared at higher temperatures, allowing them to maintain similar lipid stores as caterpillars at lower rearing temperatures. Lipids are important for flight and egg production (Wheeler 1996), and lipid stores therefore have an important impact on adult fitness.

We found no significant effect of treatment on the macronutrient content of Brussels sprouts leaves. However, we did find a significant effect of nutrient treatment on nitrogen; leaves from plants in the 4:1 treatment contained less nitrogen than those from the 16:1 and 64:1 treatments. We found the same significant trend for the nitrogen content of caterpillar frass, with



frass from the 4:1 treatment containing less nitrogen than frass from the other two treatments. In other words, when leaves contained higher amounts of nitrogen in the 64:1 treatment, more nitrogen was excreted by caterpillars in the frass. Therefore, it seems that caterpillars were not able to use higher amounts of nitrogen as efficiently. If this nitrogen had been converted to protein, perhaps the caterpillars could have used it more efficiently.

Although studies have suggested that there may be strong interactive effects of temperature and nutrients on life history traits of organisms (Cross et al. 2015), our results did not support this, with only one variable demonstrating an interactive effect. Overall, we found temperature to be the most influential variable affecting *P. rapae*. This could be because ectothermic herbivores are less able to change their response to temperature; temperature changes the rate of chemical reactions that occur within an organism, and there is no way for an animal to change that, whereas they may be able to alter aspects of their physiology, such as rate of digestion, in response to nutrient availability of plants. Although we did not find many interactive effects between nutrients and temperature, it is still important to test these variables together, as that is how organisms will be experiencing them in their natural environment.

Surprisingly we did not find an effect of temperature on the amount of consumption of caterpillars, contrary to numerous other experiments. This could be due to the method used to measure the consumption. Future experiments could be improved by also taking the dry mass of remaining uneaten leaves, instead of only estimating it from leaf area eaten by caterpillars. Another potential reason for this discrepancy is that we measured consumption over the entire larval period, whereas other experiments only used fourth instar caterpillars (Lee and Roh 2010; Bauerfeind and Fischer 2013). Our choice to measure consumption over the entire larval period may be more beneficial, as Ruusila et al. (2005) demonstrated that different larval instars may be sensitive to different plant traits, and may therefore differ in their response to plant quality depending on the instar. Additionally, different tissues from the same plant can vary in

macronutrient and elemental content (Mattson 1980), therefore it could be beneficial to provide caterpillars with multiple leaves at a time to allow them to somewhat buffer these differences. Providing caterpillars with multiple leaves could also account for potential differences in leaf mechanical traits, such as toughness. There also could also have been an influence of parental rearing conditions on the caterpillars used in this experiment; caterpillars were hatched at the same temperature and came from a colony reared at one temperature. In the future it might be beneficial to have generations from the different experimental temperatures to try to control for generational effects. Due to potential inbreeding, larvae reared from the laboratory butterfly colony may also exhibit different responses than wild caught butterflies, therefore future experiments would benefit from comparing the results of this experiment to those that use wild caught individuals. Additionally, rearing caterpillars at a lower upper temperature, such as 30°C, could result in increased plant growth and caterpillar survival, which would increase the sample size and possibly make effects more pronounced.

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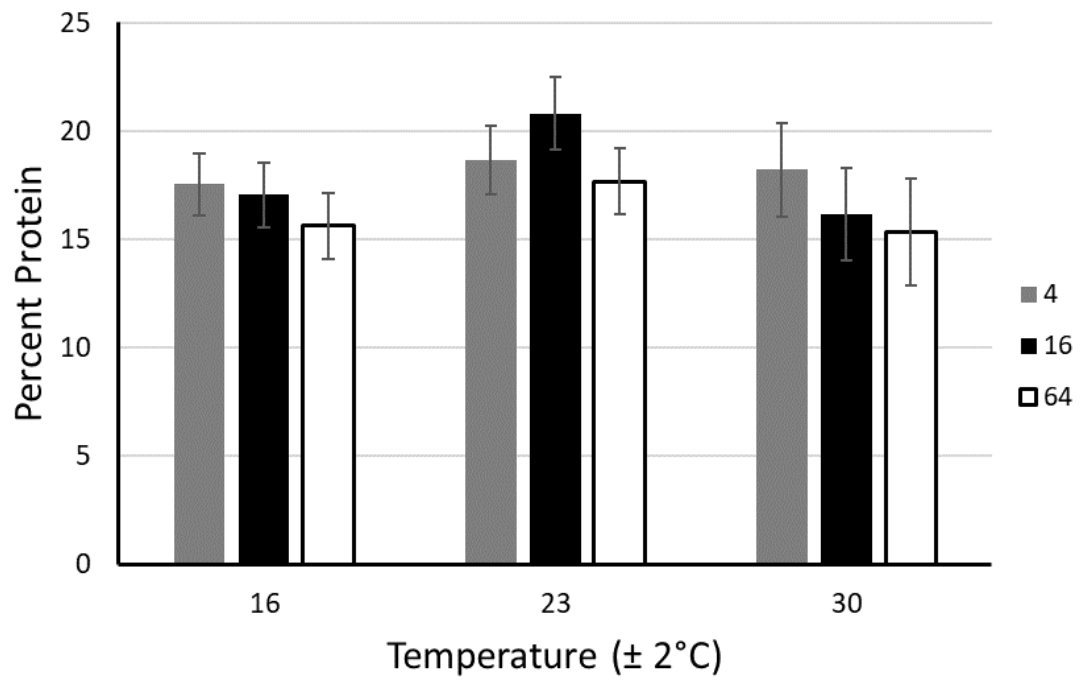
Table 1. Results of ANOVA testing the effects of nutrient and temperature treatments on macronutrients in Brussels sprouts *Brassica oleracea*. p-values < 0.05 in bold.

Dependent Variable	<i>n</i>		Nutrient Treatment ( <i>DF</i> 1)	Temperature Treatment ( <i>DF</i> 1)	Nutrient x Temperature Treatment ( <i>DF</i> 1)
Percent Protein	113	<i>F</i>	1.8	0.2	0.0006
		<i>p</i>	0.18	0.69	0.98
Percent Carbohydrate	114	<i>F</i>	0.4	1.3	0.1
		<i>p</i>	0.53	0.26	0.71
Percent Nitrogen	118	<i>F</i>	8.0	0.16	1.2
		<i>p</i>	<b>0.005</b>	0.69	0.27
Percent Carbon	118	<i>F</i>	0.2	2.0	1.6
		<i>p</i>	0.65	0.17	0.21
C:N	118	<i>F</i>	12.5	0.9	0.07
		<i>p</i>	<b>0.0006</b>	0.36	0.79

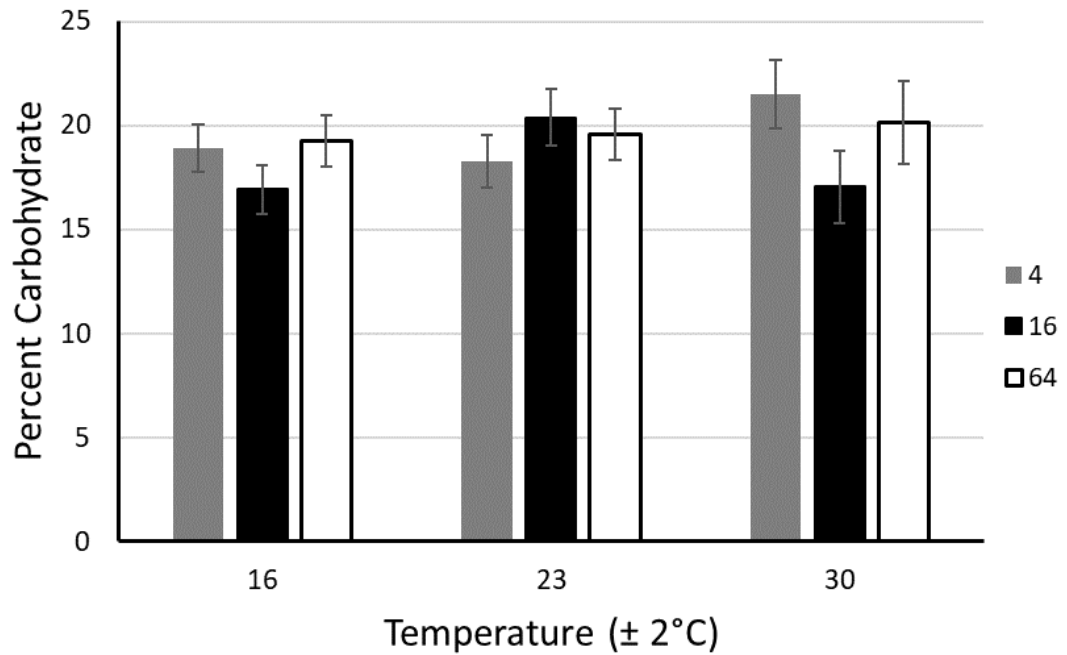
Table 2. Results of ANOVA testing the effects of nutrient and temperature treatments on life history traits of cabbage white caterpillars *Pieris rapae*. p-values < 0.05 in bold.

Dependent Variable	<i>n</i>		Nutrient Treatment ( <i>DF</i> 1)	Temperature Treatment ( <i>DF</i> 1)	Nutrient x Temperature Treatment ( <i>DF</i> 1)
Leaf Area Eaten	106	<i>F</i> <i>p</i>	3.5 0.07	0.4 0.55	1.5 0.23
Dry Mass Eaten	106	<i>F</i> <i>p</i>	3.5 0.06	0.4 0.55	1.5 0.23
Conversion Efficiency	95	<i>F</i> <i>p</i>	2.8 0.10	15.6 <b>0.0001</b>	0.1 0.73
Frass Percent Nitrogen	109	<i>F</i> <i>p</i>	6.1 <b>0.01</b>	7.8 <b>0.006</b>	0.06 0.80
Frass Percent Carbon	109	<i>F</i> <i>p</i>	0.2 0.66	0.06 0.81	2.4 0.13
Frass C:N	109	<i>F</i> <i>p</i>	2.4 0.12	6.9 <b>0.01</b>	1.0 0.32
Time to Pupation	106	<i>F</i> <i>p</i>	4.6 <b>0.03</b>	545.3 <b>&lt; 0.0001</b>	5.3 <b>0.02</b>
Pupal Duration	98	<i>F</i> <i>p</i>	0.02 0.90	397.6 <b>&lt; 0.0001</b>	0.2 0.65
Adult Wet Mass	98	<i>F</i> <i>p</i>	0.06 0.81	21.6 <b>&lt; 0.0001</b>	1.5 0.22
Adult Dry Mass	95	<i>F</i> <i>p</i>	0.07 0.80	9.5 <b>0.003</b>	0.08 0.78
Adult Wing Area	94	<i>F</i> <i>p</i>	0.001 0.97	10.4 <b>0.002</b>	0.4 0.52
Adult Percent Lipid	94	<i>F</i> <i>p</i>	23.1 <b>&lt; 0.0001</b>	3.7 0.06	1.5 0.23

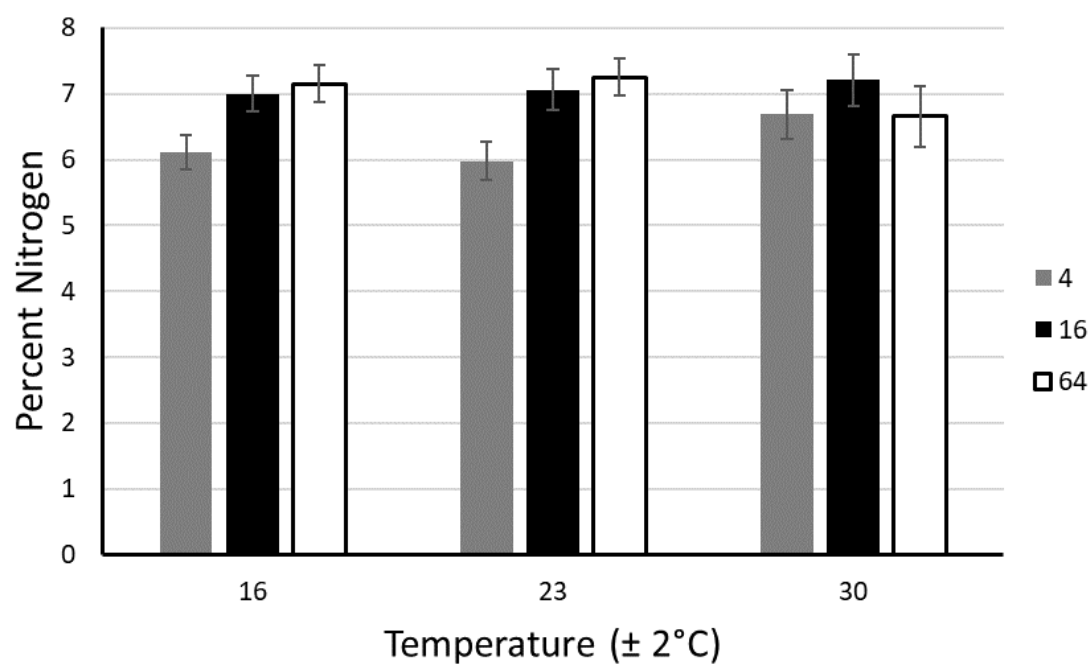




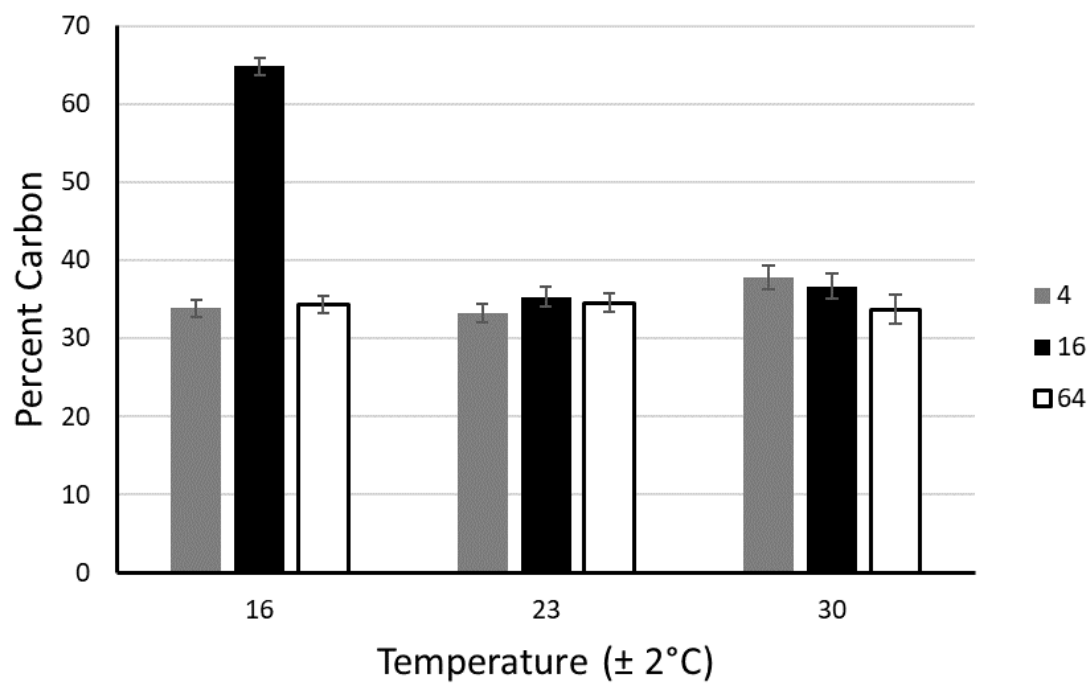
**Figure 1.** Least square means for Brussels sprout leaf percent protein ( $\pm$ SE).



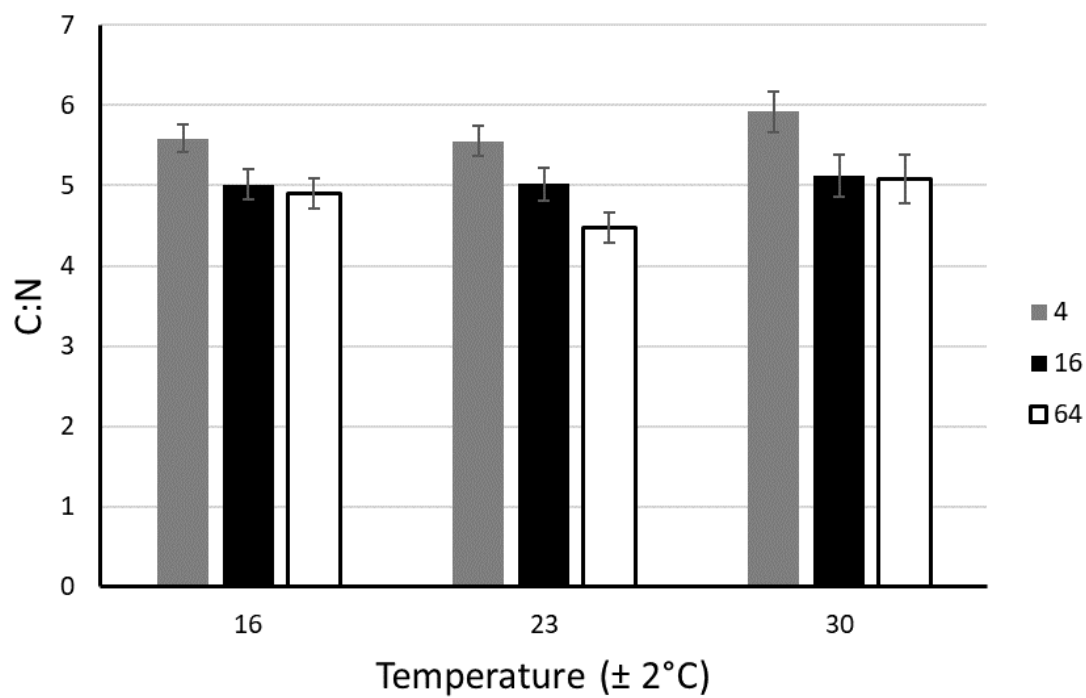
**Figure 2.** Least square means for Brussels sprout leaf percent carbohydrate composition ( $\pm$ SE).



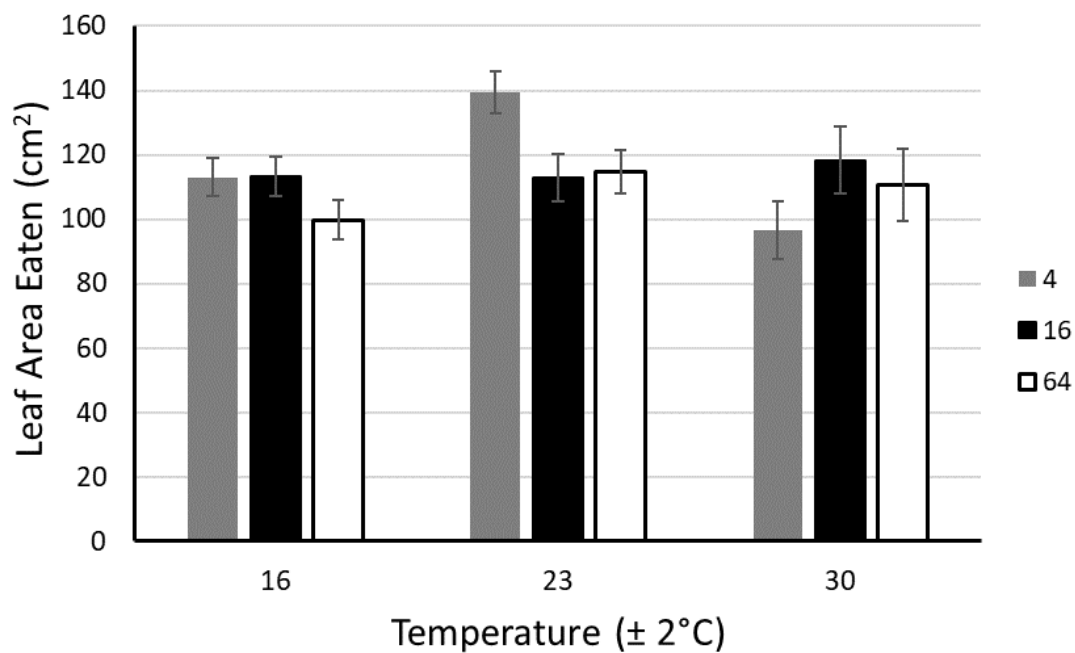
**Figure 3.** Least square means for Brussels sprout leaf percent nitrogen ( $\pm$ SE).



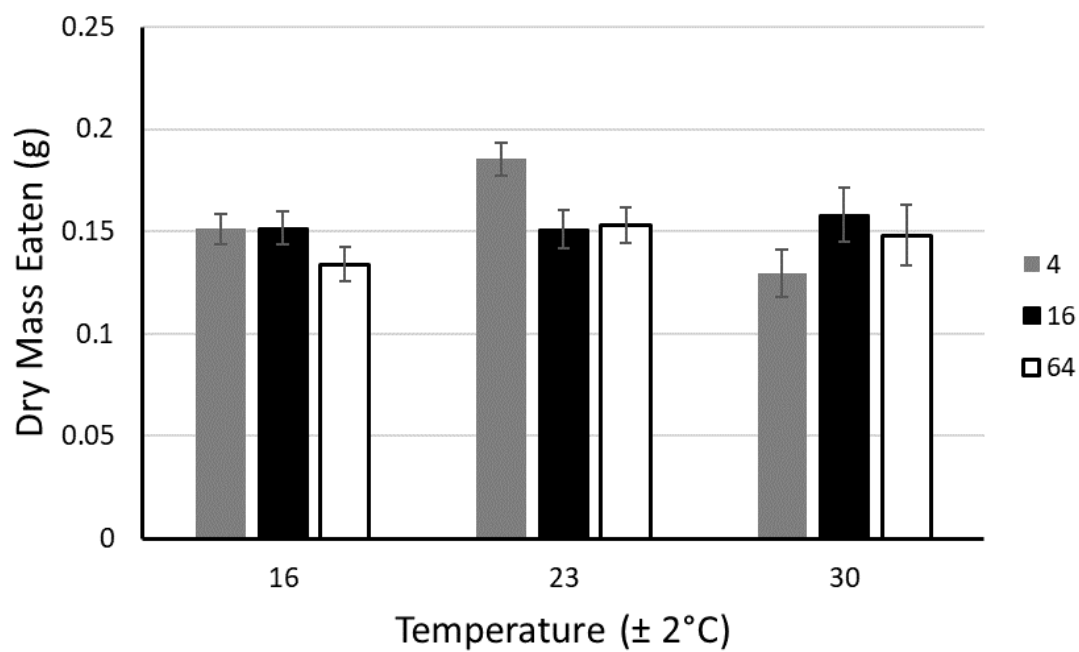
**Figure 4.** Least square means for Brussels sprout leaf percent carbon ( $\pm$ SE).



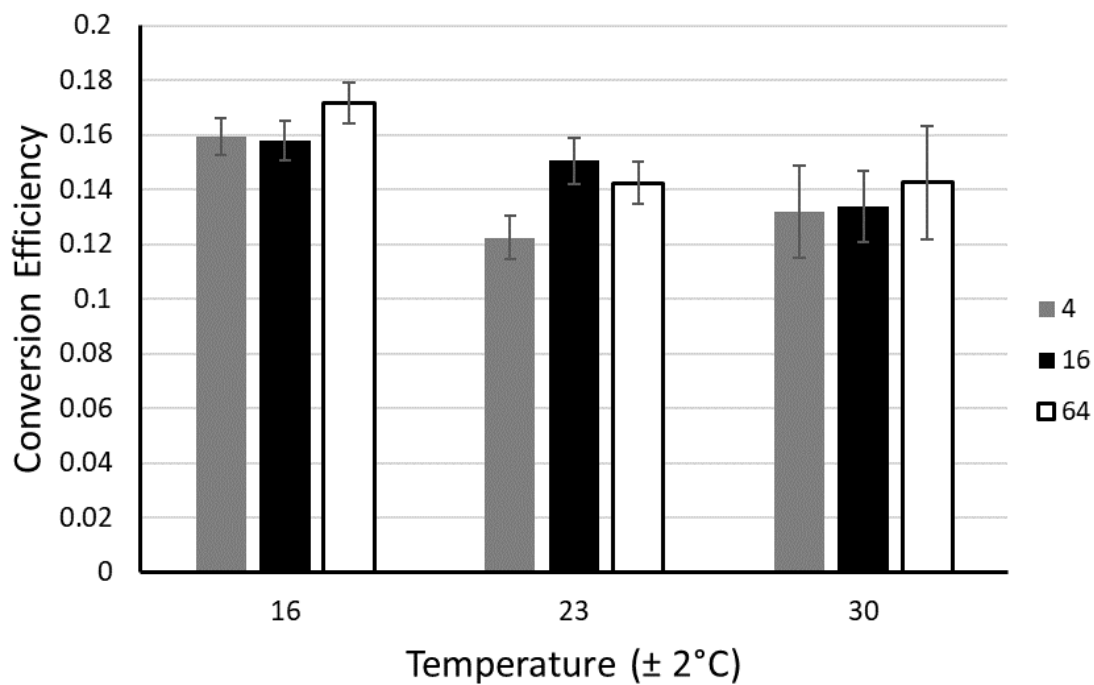
**Figure 5.** Least square means for Brussels sprout leaf percent C:N (±SE).



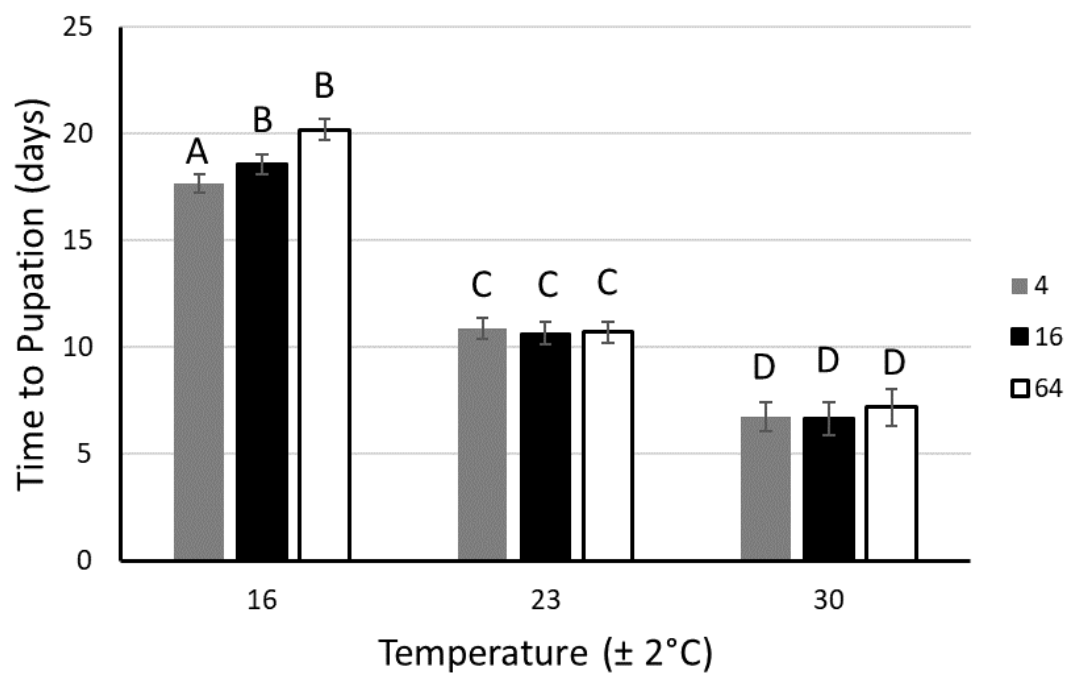
**Figure 6.** Least square means for total leaf area eaten by caterpillars until time of pupation (±SE).



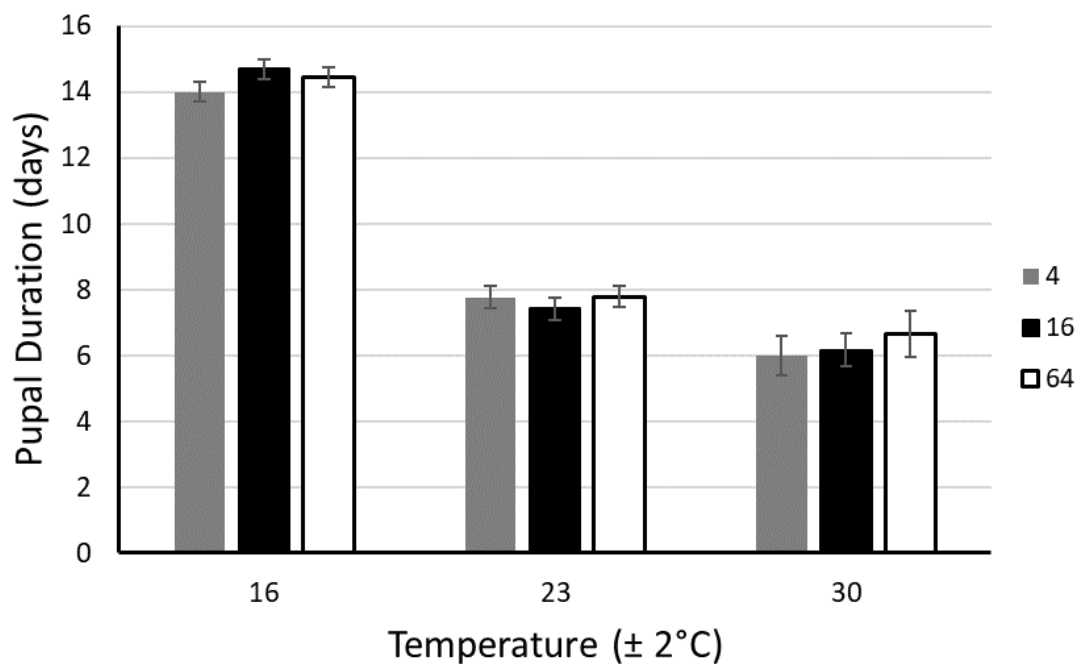
**Figure 7.** Least square means total dry mass of leaves eaten by caterpillars (±SE).



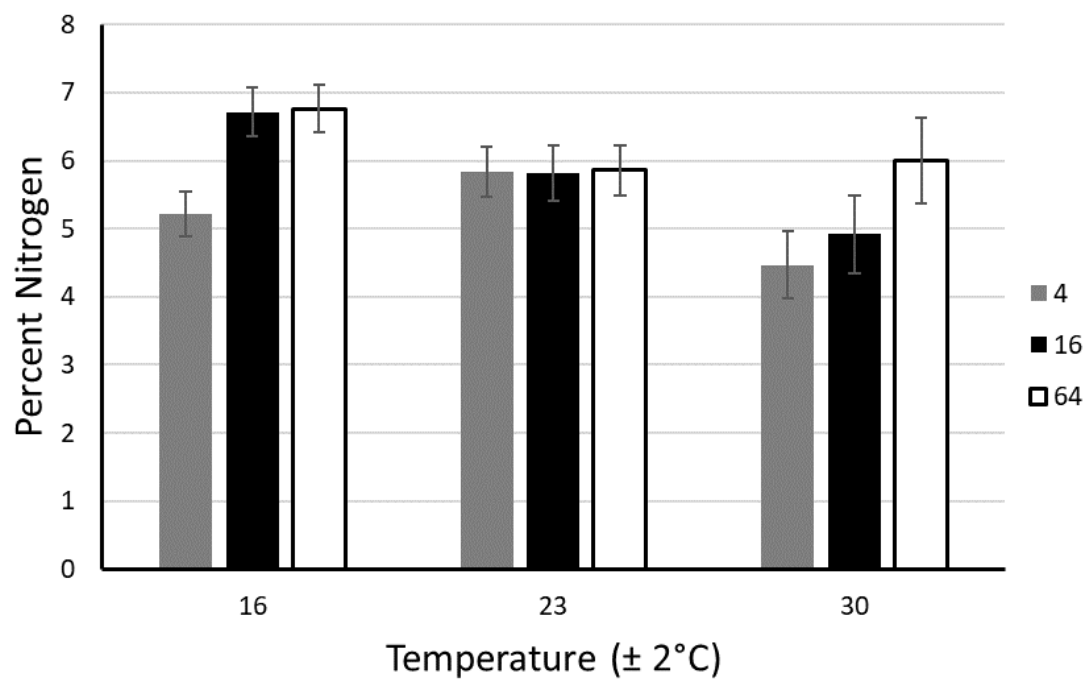
**Figure 8.** Least square means for conversion efficiency of caterpillars (±SE).



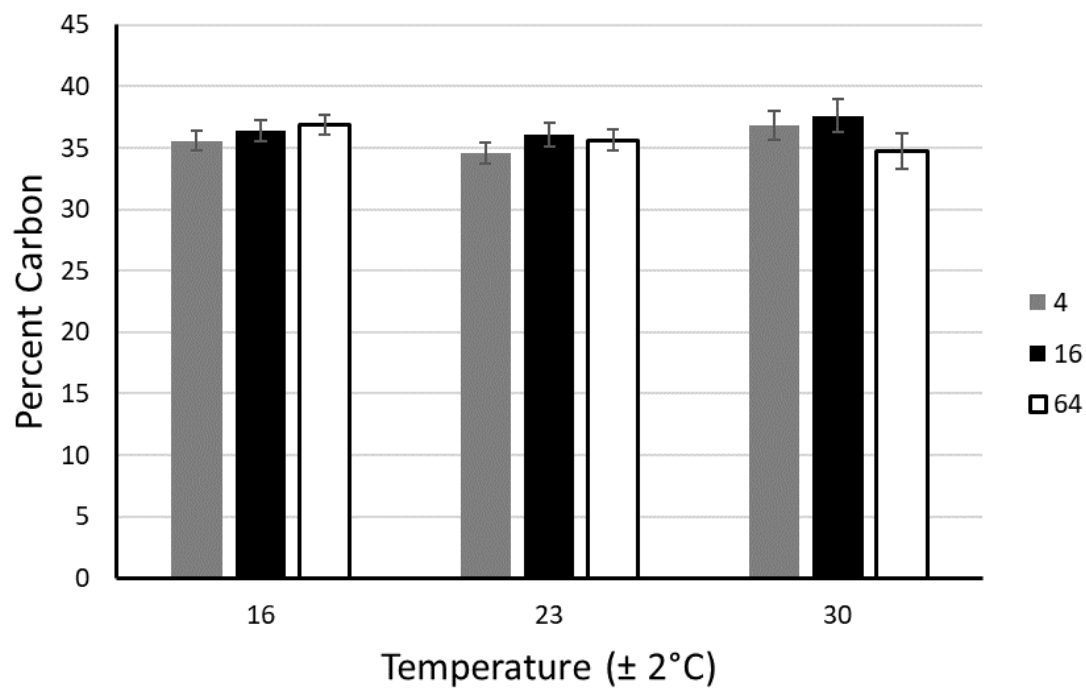
**Figure 9.** Least square means for time to pupation for caterpillars ( $\pm$ SE). Bars with different letters vary significantly according to Student's t-test.



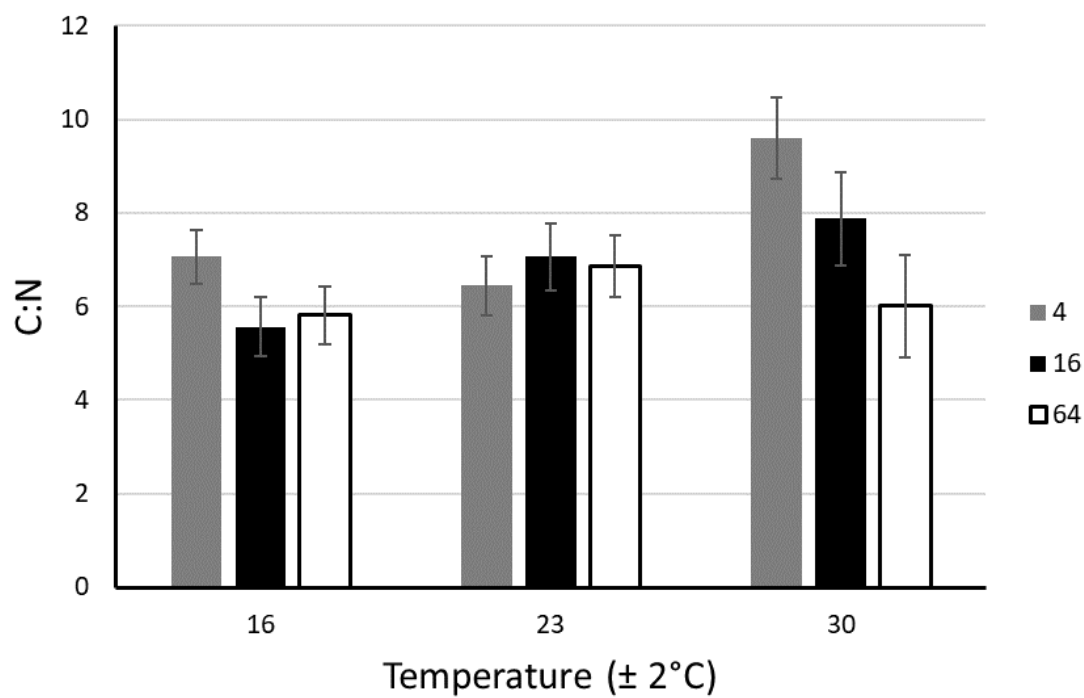
**Figure 10.** Least square means for pupal duration of caterpillars ( $\pm$ SE).



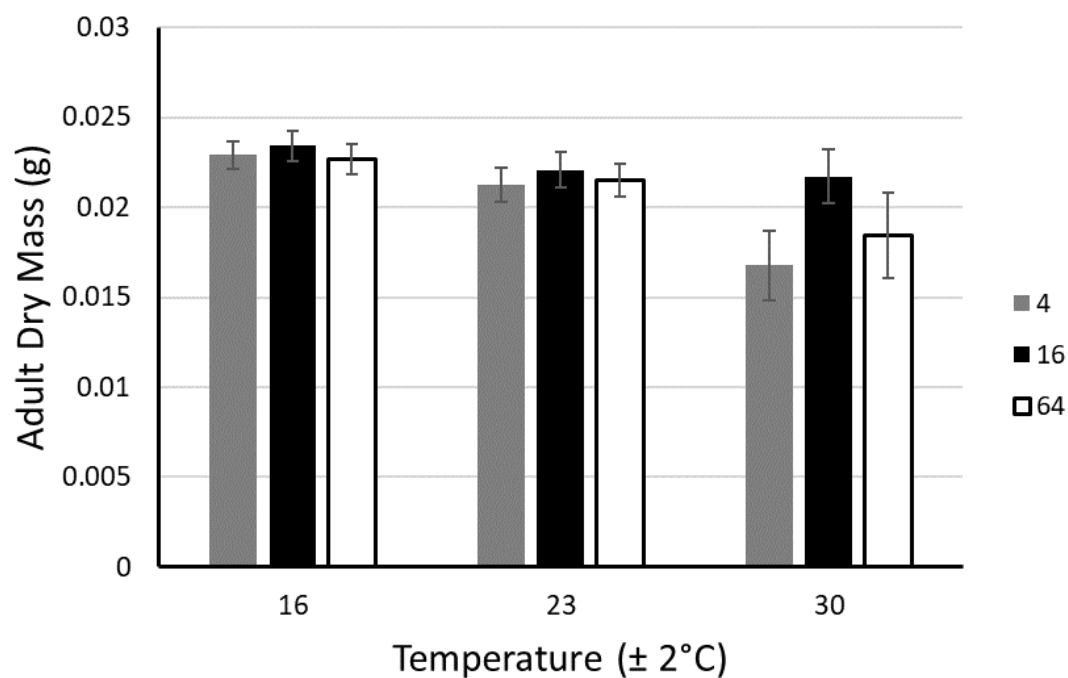
**Figure 11.** Least square means for percent nitrogen of caterpillar frass ( $\pm$ SE).



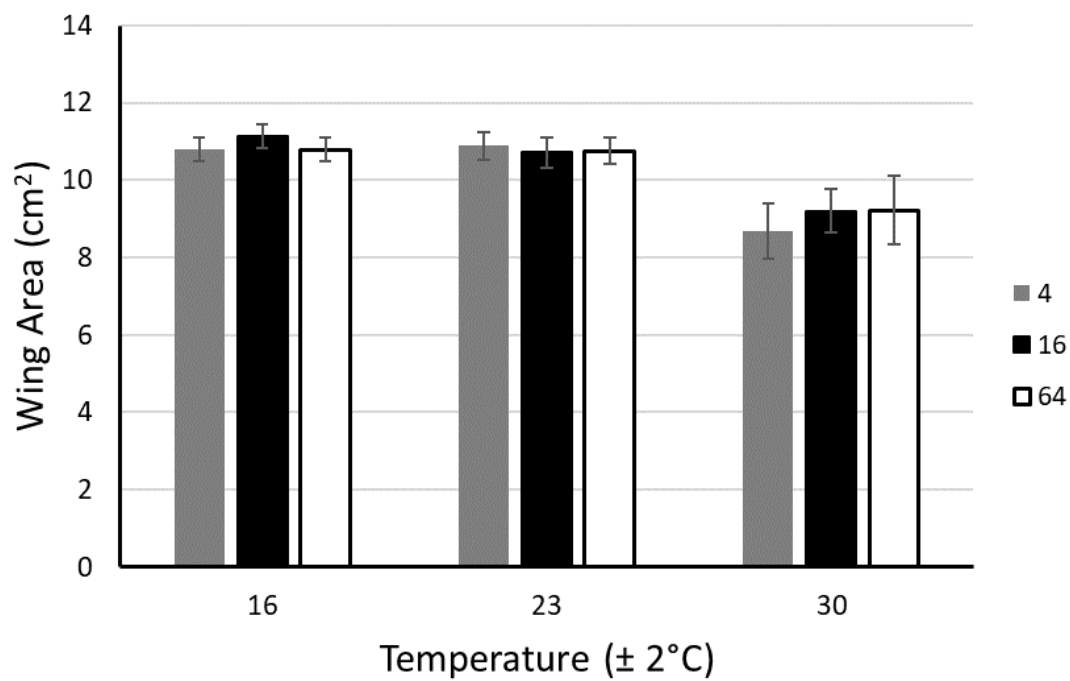
**Figure 12.** Least square means for percent carbon of caterpillar frass ( $\pm$ SE).



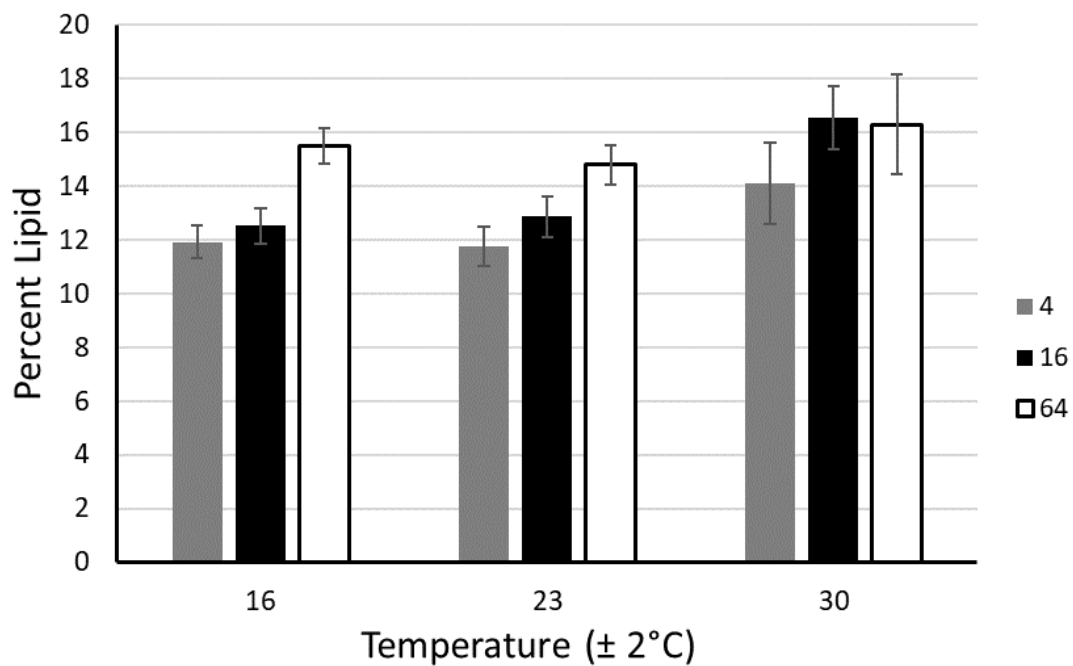
**Figure 13.** Least square means for percent C:N of caterpillar frass (±SE).



**Figure 14.** Least square means for adult butterfly dry mass (±SE).

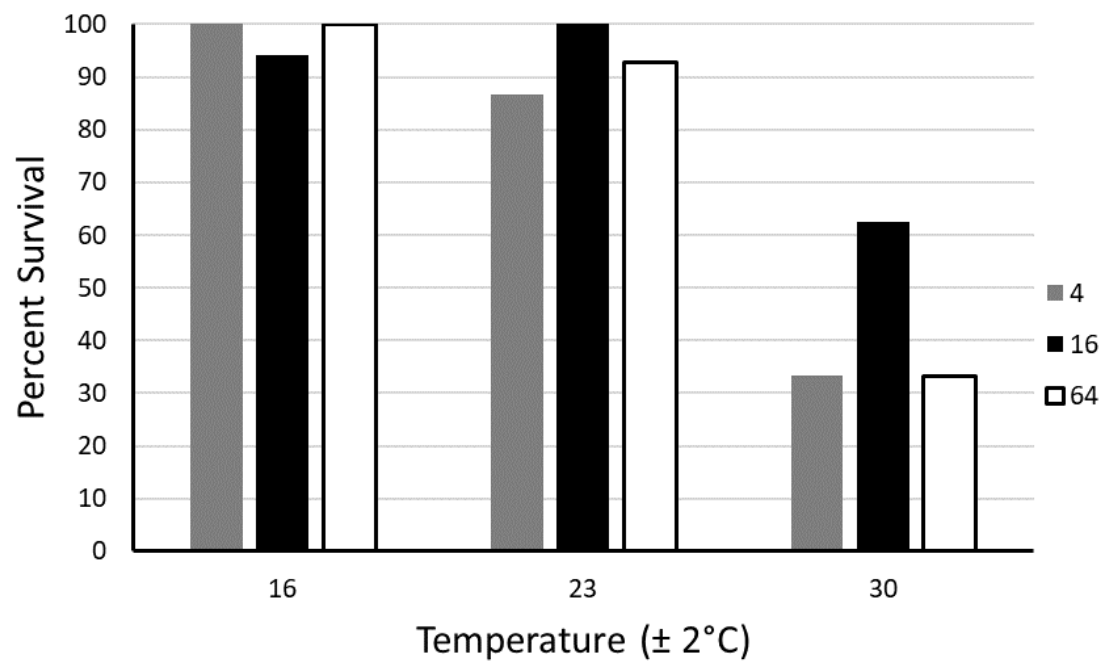


**Figure 15.** Least square means for adult butterfly wing area ( $\pm$ SE).



**Figure 16.** Least square means for adult butterfly body percent lipid composition ( $\pm$ SE).





**Figure 17.** Percentage of caterpillars that survived to emergence as adult butterflies.

VITA

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